UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

February 19, 2013

MEMORANDUM

Subject: Efficacy Review for Maguard 5626; EPA Reg. no. 10324-214; DP Barcode: D406976.

From: Ibrahim Laniyan, Ph.D.
Microbiologist
Product Science Branch
Antimicrobials Division (7510P)

Thru: Emily Mitchell
Branch Chief
Product Science Branch
Antimicrobials Division (7510P)

To: Martha Terry / Marshall Swindell PM 33
Regulatory Management Branch I
Antimicrobials Division (7510P)

Applicant: Mason Chemical Company
721 W. Algonquin Road
Arlington Heights, IL 60005

Formulation from the Label:

Active Ingredient	% by wt.
Peroxyacetic Acid	5.9 %
Hydrogen Peroxide	27.3 %
Inert Ingredients:	66.8 %
Total	100.0 %

I. BACKGROUND

The product, MAGUARD® 5626 (EPA Reg. No. 10324-214), is an approved hard non-porous surface food contact bacterial sanitizer in dairies, wineries, breweries, food and beverage plants, poultry and egg facilities and animal housing. It is also approved for hard non-porous surface disinfection [bactericide, virucide and fungicide (*Canidida albicans* and *Trichophyton mentagrophytes* only)] in hospitals, schools, industrial facilities, office buildings, and veterinary clinics and for use against odor-causing bacteria, slime, odor, algae in recirculating cooling water and evaporative coolers, in agricultural waters and in water treatment operations using reverse osmosis and nano and ultra filtration. Additionally it is designed to help control spoilage or decay-causing bacteria and fungi in raw post harvest fruits and vegetables; and is used as an antimicrobial agent in oilfield and gas field well operations, oil field water flood/salt water disposal systems and fracturing fluids. The Mason Chemical Company is submitting an amendment for this end-use product to add organisms as well as additional "Directions for Use and Marketing Claims". The label states that the product is an effective disinfectant in the presence of 400 ppm hard water and 5% blood serum. The label states that the product is an effective sanitizer when a solution is prepared in water of up to 500 ppm hardness as $CaCO_3$. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated October 18, 2012), EPA Form 8570-35 (Data Matrix), sixty four studies (MRID 489215-01 through 489215-64), Statements of No Data Confidentiality Claims for all sixty four studies, and the proposed label with lighted changes made to the label (dated 12/15/2011).

II. USE DIRECTIONS

The product is designed for use as a hard non-porous surface sanitizer, cleanser and deodorizer for the following: (1) on pre-cleaned surfaces such as equipment, pipelines, tanks, vats, filters, evaporators, pasteurizers, and aseptic equipments in dairies, breweries, wineries, beverage and food processing/packing plants, and egg processing/packing equipment surfaces; (2) as a final rinse of returnable and non-returnable bottles; and (3) on packing house conveyers, harvesting equipment and containers and associated machinery and items such as trucks, trailers, ladders, power tools, gloves and rubber boots. As a disinfectant and cleaner, the product is designed for use on hard non-porous surfaces in general use sites including animal life science laboratories, athlete/recreational areas, cruise ships, dental offices, examination rooms, food service establishments, hospitals, lodging establishments, manufacturing facilities, nursing homes, office buildings, pet shops, public restrooms, schools, transportation terminals, and veterinary clinics and in medical use sites for a large variety of uses (over 150 were listed on the label).

As a sanitizer: (1) 1 to 2 fluid ounces per 6 gallons of water for food contact surfaces with a contact time of at least 60 seconds – use immersion, coarse spray or circulation techniques as appropriate; (2) 1 to 2 fluid ounces per 5 gallons of water for batch sanitization of filtration systems – with recirculation of the solution through the piping and membrane system at 20°C for 10 minutes up to 4 hours; and (3) 2 to 5 fluid ounces per 430 gallons for reverse osmosis continuous or intermittent processing.

As a foam cleaning agent of food and non food contact surfaces: 1 to 3 fluid ounces of test product with 6 to 12 fluid ounces of Macat AO-12 (amine oxide) per 6 gallons of water for a minimum of 1 minute.

As a booster for alkaline and acidic detergents: 2 to 7 fluid ounces per gallon of detergent solution.

As a fogger: 3.5 fluid ounces per 10 gallons of water (or equivalent use dilution) at a rate of 1 quart per 1000 ft^3.

As a disinfectant/deodorizer: Either 2 fluid ounces per 1 gallon or 4 fluid ounces per 1 gallon of 400 ppm hard water and 5% blood serum, with contact times of either 2 or 5 minutes as appropriate for the application. Heavily soiled areas require a pre-cleaning step.

III. AGENCY STANDARDS FOR PROPOSED CLAIMS

Sporicidal Disinfectant against *Clostridium difficile*: The Agency has established interim guidance for the efficacy evaluation of antimicrobial products (e.g., dilutable products, ready-to-use products, spray products, vapor, gases, and towelettes) that are labeled for use to treat hard, non-porous surfaces in healthcare settings contaminated with spores of *Clostridium difficile*. The effectiveness of such a product must be substantiated by data derived from one of the following two test methods: AOAC Method 2008.05: Quantitative Three Step Method (Efficacy of Liquid Sporicides Against Spores of *Bacillus subtilis* on a Hard Nonporous Surface); and ASTM E 2197-02: Standard Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporicidal Potencies of Liquid Chemical Germicides. Modifications to each test method will be necessary to specifically accommodate spores of *Clostridium difficile*. Because *Clostridium difficile* is an obligate anaerobe, testing should ensure adequate incubation conditions for the recovery of viable spores. Three product batches should be tested at or below the lower certified limit(s) (LCL) listed on the confidential statement of formula (CSF) of the product. The toxigenic strains, ATCC 43598, of *Clostridium difficile* must be used for testing. For towelette and spray formulations, the Agency will accept testing of the liquid expressed directly from towelettes or collected directly from spray containers using one of the quantitative methods and conditions specified above. All products should be tested with a 3-part soil load incorporated into the test inoculum by adding 25 µl of 5% bovine serum albumin, 35 µl of 5% yeast extract and 100 µl of 0.4% mucin to 340 µl of the spore suspension. Results must show a minimum 6 log reduction of viable spores in 10 minutes or less. For towelette products, wetness determination test will be used to generate the contact time. Control carrier counts must be greater than 10^6 spores/carrier. The titer and purity of the final spore preparation must be $>10^8$ spores/mL, and >95% spores. ASTM Standard E2839-11 specifies procedures for achieving the 95% purity. The acid resistance of purified spores should be assessed against 2.5 M hydrochloric acid (see ASTM Standard E2839-11). The spores are considered acid-resistant if a log reduction of 0-2 is exhibited following 10 minutes of exposure to 2.5 M HCl.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments: The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old, against *Salmonella enterica* (ATCC 10708; formerly *Salmonella choleraesuis*), *Staphylococcus aureus* (ATCC 6538), and

Pseudomonas aeruginosa (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Bacteria): Effectiveness of disinfectants against specific bacteria other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products as Disinfectants Method, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different product lots. To support products labeled as "disinfectants" for specific bacteria (other than those bacteria named in the above test methods), killing of the specific microorganism on all carriers is required.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi, Using a Modified AOAC Use-Dilution Method): The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data using an appropriate test. The AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different product lots must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required.

Note: As an interim policy, EPA is accepting studies with dried carrier counts that are at least 10^4 for *Trichophyton mentagrophytes*, *Aspergillus niger*, and *Candida albicans*. EPA recognizes laboratories are experiencing problems in maintaining dried carrier counts at the 10^6 level. This interim policy will be in effect until EPA determines that the laboratories are able to achieve consistent carrier counts at the 10^6 level.

Virucides: The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Virucides – Novel Virus Protocol Standards: To ensure that a virus protocol has been adequately validated, data should be provided from at least 2 independent laboratories for each product tested (i.e., 2 product lots per laboratory).

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces): Sanitizing rinses may be formulated with quaternary ammonium compounds, chlorinated trisodium phosphate, or anionic detergent-acid formulations. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC

Germicidal and Detergent Sanitizing Action of Disinfectants Method. Data from the test on 1 sample from each of 3 different product lots, one of which is at least 60 days old against *Escherichia coli* (ATCC 11229) and *Staphylococcus aureus* (ATCC 6538) are required. When the effectiveness of the product in hard water is made, all required data must be developed at the hard water tolerance claimed. Acceptable results must demonstrate a 99.999% reduction in the number of microorganisms within 30 seconds. The results must be reported according to the actual count and the percentage reduction over the control. Furthermore, counts on the number controls for the product should fall between 75 and 125 x 10^6/mL for percent reductions to be considered valid. Label directions for use must state that a contact time of at least 1 minute is required for sanitization. A potable water rinse is not required (to remove the use solution from the treated surface) for products cleared for use on food contact surfaces under the Federal Food, Drug, and Cosmetic Act. Label directions must recommend a potable water rinse (to remove the use solution from the treated surface) under any other circumstances.

Sanitizers (For Non-Food Contact Surfaces; Additional Bacteria): There are cases where an applicant requests to make claims of effectiveness against additional microorganisms for a product that is to be used as a sanitizer for non-food contact surfaces. Confirmatory test standards would apply. Therefore, 2 product samples, representing 2 different product lots, should be tested against each additional microorganism. The ASTM method states that the inoculum employed should provide a count of at least 7.5 x 10^5 colony forming units per carrier. Results must show a bacterial reduction of at least 99.9 percent over the parallel control within 5 minutes.

Supplemental Claims: An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum. On a product label, the hard water tolerance level may differ with the level of antimicrobial activity (e.g., sanitizer vs. disinfectant) claimed. To establish efficacy in hard water, all microorganisms (i.e., bacteria, fungi, and viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

IV. BRIEF DESCRIPTION OF THE DATA

1. MRID 489215-01: "AOAC Use-Dilution Method, Test Organism: *Acinetobacter baumannii* (Multi-drug Resistant) (ATCC 19606)" for Maguard 5626, Lot 1621-381, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 9, 2012. Project Number A13748.

This study was conducted against *Acinetobacter baumannii* (Multi-drug Resistant) (ATCC 19606). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.12 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 1.9 oz/gallon, defined as 1 part test substances + 66 parts of 400 ppm AOAC synthetic hard water. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The age of product Lot 1621-381 was at least 60 days old at the time of testing. Ten (10) stainless steel penicylinders were immersed for 15±2 min in the culture suspension at a ratio of 1 carrier per 1 mL of culture. The carriers were dried for 38 min at 35-37°C and 50% relative humidity. At staggered intervals, the contaminated and dried carriers were transferred by hook needle to individual tubes containing 10 mL of the test product at the appropriate test dilution. The carriers were exposed for 2 minutes at 20±1°C. Each medicated carrier was then transferred by wire hook, at identical staggered intervals to 10

mL of Letheen Broth + 0.1% of sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier sterility, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Multi-drug Resistant *Acinetobacter baumannii* (ATCC 19606) was verified on a representative culture in tests conducted by the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. Results of these tests are presented in Attachment I on page 17 of 27 in MRID 489215-01; the test method is not described.

Note: Protocol amendment reported in the study (change of test species name) was reviewed.

2. MRID 489215-02: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 5 (ATCC VR-5, Strain Adenoid 75)" for Maguard 5626, Batch ZC, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – May 9, 2012. Project Number A13099.

This study was conducted against Adenovirus type 5 (ATCC VR-5, Strain Adenoid 75), using A-549 cells obtained from American Type Culture Collection (ATCC CCL-185) as the infectivity assay. One lot (Batch ZC) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03041112.ADV (copy provided). The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 4 parts test substance + 124 parts of 400 ppm AOAC Synthetic Hard Water. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 200 µl of virus inoculum uniformly over the bottom of separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). Five dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for two minutes at room temperature (20°C). The virus films were completely covered with the use solution. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixtures were passed through individual Sephadex columns. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. The A-549 cell line which exhibits cytopathic effect (CPE) in the presence of Adenovirus type 5 was used as the indicator cell line in the infectivity assays. Cells in multi-well culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from the test and control groups. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10 days for the presence or absence of CPE, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

3. MRID 489215-03: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Adenovirus type 5 (ATCC VR-5, Strain Adenoid 75)" for Maguard 5626, Lot 1621-381, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – July 31, 2012. Project Number A13695.

This study was conducted against Adenovirus type 5 (ATCC VR-5, Strain Adenoid 75), using A-549 cells obtained from American Type Culture Collection (ATCC CCL-185) as the infectivity assay. One lot (#1621-381) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03062512.ADV.1 (copy provided). The product was received as a

concentrated solution and was diluted at a rate 1:32, 1 part test substance + 31 parts of 400 ppm AOAC Synthetic Hard Water. The stock virus culture was adjusted to contain a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 200 µl of virus inoculum uniformly over the bottom of separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). Five dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for two minutes at room temperature (20°C). The virus films were completely covered with the use solution. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixtures were passed through individual Sephadex columns. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. The A-549 cell line which exhibits cytopathic effect (CPE) in the presence of Adenovirus type 5 was used as the indicator cell line in the infectivity assays. Cells in multi-well culture dishes were inoculated in quadruplicate with 100 µL of the dilutions prepared from the test and control groups. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 11 days for the presence or absence of CPE, cytotoxicity, and viability. Controls included those for dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

4. MRID 489215-04: "AOAC Use-Dilution Method, Test Organism: *Acinetobacter baumannii (Multi-drug Resistant)* (ATCC 19606)" for Maguard 5626, Lot 1621-382, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13749.

This study was conducted against *Acinetobacter baumannii* (Multi-drug Resistant) (ATCC 19606). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.28 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 1.9 oz/gallon, defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The age of product Lot 1621-382 was at least 60 days old at the time of testing. Ten (10) stainless steel penicylinders were immersed for 15±2 min in the culture suspension at a ratio of 1 carrier per 1 mL of culture. The carriers were dried for 38 min at 35-37°C and 50% relative humidity. At staggered intervals, the contaminated and dried carriers were transferred by hook needle to individual tubes containing 10 mL of the test product at the appropriate test dilution. The carriers were exposed for 2 minutes at 20±1°C. Each medicated carrier was then transferred by wire hook, at identical staggered intervals to 10 mL of Letheen Broth + 0.1% of sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, organic soil sterility, carrier sterility, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Multi-drug Resistant *Acinetobacter baumannii* (ATCC 19606) was verified on a representative culture in tests conducted by the University of Minnesota Physicians Outreach Laboratory in Minneapolis, MN. Results of these tests are presented in Attachment I on page 17 of 27 in MRID 489215-04; the test method is not described.

Note: Protocol amendment reported in the study (change of test species name) was reviewed.

Note: The neutralization confirmation control that was run concurrently did not meet the acceptance criteria. The control was repeated post-test and met the criteria for acceptance.

mL of the use solution for 2 minutes at 20 ± 1 °C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures and control plates were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for culture purity, organic soil sterility, carrier sterility, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. Negative control and positive control agars were prepared using respectively *Escherichia coli* (ATCC 35218) and *Klebsiella pneumoniae* (ATCC 700603). Two Etest strips, one containing Cefotaxime (CT) and Cefotaxime+Clavulanic acid (CTL) and the other Ceftazidime (TZ) and Ceftazidime+Clavulanic acid (TZL) were placed on each plate. The plates were incubated and, following incubation, the Minimum Inhibitory Concentration (MIC) was read. The measurement confirmed production of *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196). See Table 5 on pages 17 of the laboratory report.

17. MRID 489215-17 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Escherichia coli* O26:H11 (ATCC BAA-1653)" for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 3, 2012. Project Number A13713.

This study was conducted against *Escherichia coli* O26:H11 (ATCC BAA-1653). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03061112.GDST.19 (copy provided). The use solution (a 1:768 dilution of the product or 1 oz/6 gal) was prepared by adding 1 oz of the product and 767 oz of 500 ppm AOAC synthetic hard water (titrated value was 502 ppm). The absorbance value of the culture suspension was measured (at 620 nm) using a spectrophotometer; the absorbance value was determined to be 2.010. Use solutions were not tested in the presence of a 5% organic soil load. 99.0-mL aliquots of the use solution were transferred to duplicate 250-300 mL Erlenmeyer flasks, placed in a water bath at 25 ± 1°C and allowed to equilibrate for ≥20 min. One-mL bacterial suspensions were added to each flask and exposed for 30 sec at 25 ± 1 °C. One-mL aliquots of the bacterium-product mixture were then transferred to 9 mL of Letheen Broth containing 0.07% lecithin and 0.5% Tween 80. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptone glucose extract agar. All plates were incubated for 48 ± 4 hours at 35-37°C, then stored at 2-8 °C for 2 days prior to reading. Controls included those for population, purity, neutralizer sterility, diluent sterility, PBDW (phosphate buffer dilution water) sterility, subculture agar sterility, viability, and neutralization confirmation.

18. MRID 489215-18 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 2," for Maguard 5626, by Dawn Pierson. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13707.

This study was conducted against Herpes simplex virus type 2 (Strain G, ATCC VR-734), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two batches of Maguard 5626 (Batch 1621-381 and Batch 1621-382) were tested according to ATS Labs Protocol No. MC03062512.HSV2.1 (copy provided). The two batches were each prepared as a 1.9 oz/gallon

tryptone glucose extract agar. All plates were incubated for 48 ± 4 hours at 35-37°C prior to reading. Controls included those for population, purity, neutralizer sterility, diluent sterility, PBDW (phosphate buffer dilution water) sterility, subculture agar sterility, viability, and neutralization confirmation.

15. MRID 489215-15 "AOAC Use-Dilution Method, Test Organism: *Escherichia coli*- with extended beta-lactamase resistance (ESBL) (ATCC BAA-196)," for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 2, 2012. Project Number A13733.

This study was conducted against *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.UD.15 (copy provided). Antimicrobial susceptibility testing was performed to verify the antimicrobial resistance. The product was diluted using 1 part test material + 66 parts 400 ppm AOAC synthetic hard water (1:67; titrated value was 401 ppm). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15 ± 2 minutes in a suspension of test organism, at a ratio of 1 carrier per 1 mL culture. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20 ± 1 °C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures and control plates were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for culture purity, organic soil sterility, carrier sterility, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. Negative control and positive control agars were prepared using respectively *Escherichia coli* (ATCC 35218) and *Klebsiella pneumoniae* (ATCC 700603). Two Etest strips, one containing Cefotaxime (CT) and Cefotaxime+Clavulanic acid (CTL) and the other Ceftazidime (TZ) and Ceftazidime+Clavulanic acid (TZL) were placed on each plate. The plates were incubated and, following incubation, the Minimum Inhibitory Concentration (MIC) was read. The measurement confirmed production of *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196). See Table 5 on pages 17 of the laboratory report.

16. MRID 489215-16 "AOAC Use-Dilution Method, Test Organism: *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196)," for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 2, 2012. Project Number A13735.

This study was conducted against *Escherichia coli* with extended beta-lactamase resistance (ESBL) (ATCC BAA-196). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.UD.25 (copy provided). Antimicrobial susceptibility testing was performed to verify the antimicrobial resistance. The product was diluted using 1 part test material + 66 parts 400 ppm AOAC synthetic hard water (1:67; titrated value was 401 ppm). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15 ± 2 minutes in a suspension of test organism, at a ratio of 1 carrier per 1 mL culture. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0

determined to be 1.865. Use solutions were not tested in the presence of a 5% organic soil load. 99.0-mL aliquots of the use solution were transferred to duplicate 250-300 mL Erlenmeyer flasks, placed in a water bath at 25.0°C and allowed to equilibrate for ≥20 min. One-mL bacterial suspensions were added to each flask and exposed for 30 sec at 25 ± 1 °C. One-mL aliquots of the bacterium-product mixture were then transferred to 9 mL of Letheen Broth containing 0.07% lecithin and 0.5% Tween 80. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptone glucose extract agar. All plates were incubated for 48 ± 4 hours at 35-37°C prior to reading. Controls included those for population, purity, neutralizer sterility, diluent sterility, PBDW (phosphate buffer dilution water) sterility, subculture agar sterility, viability, and neutralization confirmation.

13. MRID 489215-13 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Escherichia coli* O121:K-:H10 (ECL 39W)," for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 15, 2012. Project Number A13746.

This study was conducted against *Escherichia coli* O121:K-:H10 (ECL 39W). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Laboratory Protocol No. MC03061112.GDST.17 (copy provided). The use solution (a 1:768 dilution of the product) was prepared by adding 1 oz of the product and 767 oz of 500 ppm AOAC synthetic hard water (1.67; titrated value was 494 ppm). The absorbance value of the culture suspension was measured at 620 nm using a spectrophotometer and determined to be 1.970. Use solutions were not tested in the presence of a 5% organic soil load. 99.0-mL aliquots of the use solution were transferred to duplicate 250-300 mL Erlenmeyer flasks, placed in a water bath at 25 ± 1°C and allowed to equilibrate for ≥20 min. One-mL bacterial suspensions were added to each flask and exposed for 30 sec at 25 ± 1°C. One-mL aliquots of the bacterium-product mixture were then transferred to 9 mL of Letheen Broth containing 0.07% lecithin and 0.5% Tween 80. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptone glucose extract agar. All plates were incubated for 48 ± 4 hours at 35-37°C prior to reading. Controls included those for population, purity, neutralizer sterility, diluent sterility, PBDW (phosphate buffer dilution water) sterility, subculture agar sterility, viability, and neutralization confirmation.

14. MRID 489215-14 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Escherichia coli* O45:K-:H-ECL (ECL 1001)," for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 7, 2012. Project Number A13773.

This study was conducted against *Escherichia coli* O45:K-:H-ECL (ECL 1001). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Laboratory Protocol No. MC03061112.GDST.20 (copy provided). The use solution (a 1:768 dilution of the product) was prepared by adding 1 oz of the product and 767 oz of 500 ppm AOAC synthetic hard water (titrated value was 498 ppm). The culture suspension was adjusted to target *ca.* 1×10^{10} CFU/mL. The absorbance value of the culture suspension was measured at 620 nm using a spectrophotometer and determined to be 1.869. Use solutions were not tested in the presence of a 5% organic soil load. 99.0-mL aliquots of the use solution were transferred to duplicate 250-300 mL Erlenmeyer flasks, placed in a water bath at 25 ± 1°C and allowed to equilibrate for ≥20 min. One-mL bacterial suspensions were added to each flask and exposed for 30 sec at 25 ± 1°C. One-mL aliquots of the bacterium-product mixture were then transferred to 9 mL of Letheen Broth containing 0.07% lecithin and 0.5% Tween 80. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in

was prepared using 2 mL of the test substance per 132.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 40% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus*- CA-MRSA Genotype USA 400 (NRS 123) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus*- CA-MRSA Genotype USA 400 (NRS 123) to oxacillin.

11. MRID 489215-11 "AOAC Use-Dilution Method, Test Organism: *Escherichia coli* (ATCC 11229)" for Maguard 5626, by Becky Lien. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13796.

This study was conducted against *Escherichia coli* (ATCC 11229). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03062512.UD.9 (copy provided). The product was diluted using 1 part test material + 66 parts 400 ppm AOAC synthetic hard water (1:67; titrated value was 401 ppm). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15 ± 2 minutes in a suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 19.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% sodium thiosulfate to neutralize. All subcultures and control plates were incubated for 48 ± 2 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for culture purity, organic soil sterility, carrier sterility, neutralizing subculture medium sterility, viability, neutralization confirmation, and carrier population.

12. MRID 489215-12 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Escherichia coli* O103:K.:H8 (ATCC 23982)" for Maguard 5626, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13745.

This study was conducted against *Escherichia coli* O103:K.:H8 (ATCC 23982). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03061112.GDST.16 (copy provided). The use solution (a 1:768 dilution of the product or 1 oz/6 gal) was prepared by adding 1 oz of the product and 767 oz of 500 ppm AOAC synthetic hard water (1:768; titrated value was 498 ppm). The culture suspension was adjusted to target a 1×10^{10} CFU/mL culture suspension. The absorbance value of the culture suspension was measured (at 620 nm) using a spectrophotometer; the absorbance value was

5. MRID 489215-05: "AOAC Use-Dilution Method, Test Organism: *Bordetella pertussis* (ATCC 12743) for Maguard 5626, by Becky Lein. Study conducted at ATS Labs. Study completion date – August 15, 2012. Project Number A13810.

This study was conducted against *Bordetella pertussis* (ATCC 12743). Two lots (Lot No. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03062512.UD.8 (copy provided). Based on the experimental start date, both lots were ≥60 days old. The product was prepared using 1 part of test substance and 66 parts of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers per lot were immersed for 15±2 minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 66% relative humidity, for 38 minutes at 25-30°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 19°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 3 days at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

6. MRID 489215-06: "Standard Quantitative Disk Carrier Test Method, Test Organism: *Clostridium difficile,* spore form (ATCC 43598)" for Maguard 5626, Batch ZC, by Anne Stemper. Study conducted at ATS Labs. Study completion date – March 22, 2012. Project Number A12888.

This study was conducted against *Clostridium difficile,* spore form (ATCC 43598). One lot of the product (Batch ZC), Maguard 5626, was tested using ATS Labs protocol # MC03021712.QDCT.2 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 1 part test solution to 31 parts of 400 ppm AOAC synthetic hard water (1:32). Up to 20 sterile carriers were transferred to individual sterile petri dishes matted with filter paper. Ten (10) µL of culture was placed in the center of each disk. After all disks in each petri dish were inoculated, the dishes were covered and the contaminated carriers were placed in a desiccator containing active desiccant. A vacuum was drawn and the carriers were dried for 7 hours under ambient conditions. Only carriers not showing signs of run-off were used in the tests. The contaminated and dried carriers were placed into separate sterile 15 mL flat-bottomed Teflon QCT vials with the contaminated side facing up. Fifty (50) µL of test substance at its use dilution was applied to the center of each disk. The test substance was allowed to remain in contact with the disk for 2 minutes at room temperature (20°C) and 22% relative humidity. Following the 2-min exposures, 10 mL of neutralizer (10 mL of Letheen Broth + 0.1% of sodium thiosulfate + 0.01% catalase) was added to each vial containing the carriers. The corresponding HCl control consisted of a modified fluid thioglycollate medium. The surface of the carriers were scraped, the vials containing the carriers were vortex mixed and the contents transferred to separate filter membranes with 0.45 µm porosity. The vials were rinsed and vortex-mixed three separate times and each time the rinse solutions were transferred to the same filter membranes. The contents were evacuated, after which each filter membrane was removed aseptically from the filter unit and placed on the surface of an agar plate (CCFA-HT Agar) for recovery of *C. difficile* spores. The subcultures were incubated for 48±4 hr at 35-37°C, then stored for 2 days at 2-8°C prior to spore enumeration. Controls included those for culture purity, carrier sterility, neutralizer sterility, initial suspension population, neutralization confirmation, carrier population, and HCl resistance.

7. MRID 489215-07: "Standard Quantitative Disk Carrier Test Method, Test Organism: *Clostridium difficile,* spore form (ATCC 43598)" for Maguard 5626, Lot 1621-381 (≥60 days old), by Anne Stemper. Study conducted at ATS Labs. Study completion date – June 13, 2012. Project Number A13392.

This study was conducted against *Clostridium difficile,* spore form (ATCC 43598). One lot of the product (Lot 1621-381, ≥60 days old), Maguard 5626, was tested using ATS Labs protocol # MC03051412.QDCT.1 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 1 part test solution to 31 parts of 400 ppm AOAC synthetic hard water (1:32). The product Lot # 1621-381 was at least 60 days old at the time of testing. Brushed stainless steel carriers were used in the test. Up to 20 sterile carriers were transferred to individual sterile petri dishes matted with filter paper. Ten (10) µL of culture was placed in the center of each disk. After all disks in each petri dish were inoculated, the dishes were covered and the contaminated carriers were placed in a desiccator containing active desiccant. A vacuum was drawn and the carriers were dried for 2 hours 38 minutes under ambient conditions. Only carriers not showing signs of run-off were used in the tests. The contaminated and dried carriers were placed into separate sterile 15 mL flat-bottomed Teflon QCT vials with the contaminated side facing up. Fifty (50) µL of test substance at its use dilution was applied to the center of each disk. The test substance was allowed to remain in contact with the disk for 2 minutes at room temperature (21°C) and 49% relative humidity. Following the 2-min exposures, 10 mL of neutralizer (10 mL of Letheen Broth + 0.1% of sodium thiosulfate + 0.01% catalase) was added to each vial containing the carriers. The corresponding HCl control consisted of a modified fluid thioglycollate medium. The surface of the carriers was scraped, the vials containing the carriers were vortex-mixed and the contents transferred to separate filter membranes with 0.45 µm porosity. The vials were rinsed and vortex-mixed three separate times and each time the rinse solutions were transferred to the same filter membranes. The contents were evacuated, after which each filter membrane was removed aseptically from the filter unit and placed on the surface of an agar plate (CCFA-HT Agar) for recovery of *C. difficile* spores. The subcultures were incubated anaerobically for 48±4 hr at 35-37°C prior to spore enumeration. Controls included those for culture purity, carrier sterility, neutralizer sterility, initial suspension population, neutralization confirmation, carrier population, and HCl resistance.

8. MRID 489215-08: "Standard Quantitative Disk Carrier Test Method, Test Organism: *Clostridium difficile* - spore form (ATCC 43598)" for Maguard 5626, Lot 1621-382 (≥60 days old), by Anne Stemper. Study conducted at ATS Labs. Study completion date – June 19, 2012. Project Number A13393.

This study was conducted against *Clostridium difficile,* spore form (ATCC 43598). One lot of the product (Lot 1621-382, ≥60 days old), Maguard 5626, was tested using ATS Labs protocol # MC03051412.QDCT.2 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 1 part test solution to 31 parts of 400 ppm AOAC synthetic hard water (1:32). The product Lot # 1621-382 was at least 60 days old at the time of testing (see note below). Brushed stainless steel carriers were used in the test. Up to 20 sterile carriers were transferred to individual sterile petri dishes matted with filter paper. Ten (10) µL of culture was placed in the center of each disk. After all disks in each petri dish were inoculated, the dishes were covered and the contaminated carriers were placed in a desiccator containing active desiccant. A vacuum was drawn and the carriers were dried for 2 hours 38 minutes under ambient conditions. Only carriers not showing signs of run-off were used in the tests. The contaminated and dried carriers were placed into separate sterile 15 mL flat-bottomed Teflon QCT vials with the contaminated side facing up. Fifty (50) µL of test substance at its use dilution

was applied to the center of each disk. The test substance was allowed to remain in contact with the disk for 2 minutes at room temperature (21°C) and 49% relative humidity. Following the 2-min exposures, 10 mL of neutralizer (10 mL of Letheen Broth + 0.1% of sodium thiosulfate + 0.01% catalase) was added to each vial containing the carriers. The corresponding HCl control consisted of a modified fluid thioglycollate medium. The surface of the carriers was scraped, the vials containing the carriers were vortex-mixed and the contents transferred to separate filter membranes with 0.45 µm porosity. The vials were rinsed and vortex-mixed four separate times and each time the rinse solutions were transferred to the same filter membranes. The contents were evacuated, after which each filter membrane was removed aseptically from the filter unit and placed on the surface of an agar plate (CCFA-HT Agar) for recovery of *C. difficile* spores. The subcultures were incubated anaerobically for 48±4 hr at 35-37°C prior to spore enumeration. Controls included those for culture purity, carrier sterility, neutralizer sterility, initial suspension population, neutralization confirmation, carrier population, and HCl resistance. All data measurements for these controls were within acceptance criteria.

9. MRID 489215-09: "AOAC Use-Dilution Method, Test Organisms: Community Acquired Methicillin Resistant *Staphylococcus aureus* [CA-MRSA Genotype USA 400 (NRS 123)]" for Maguard 5626 by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 15, 2012. Project Number A13718.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 400 (NRS 123). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.3 (copy provided). The product was prepared using 2 mL of the test substance per 132.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 40% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus*- CA-MRSA Genotype USA 400 (NRS 123) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Community Acquired Methicillin Resistant *Staphylococcus aureus*- CA-MRSA Genotype USA 400 (NRS 123) to oxacillin.

10. MRID 489215-10: "AOAC Use-Dilution Method, Test Organisms: Community Acquired Methicillin Resistant *Staphylococcus aureus* [CA-MRSA Genotype USA 400 (NRS 123)]" for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13720.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* - CA-MRSA Genotype USA 400 (NRS 123). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.18 (copy provided). The product

dilution, defined as 1.00 mL product + 66.0 mL (1:67) of 400 ppm AOAC synthetic hard water (1:67) and were used on the day of preparation. The prepared test substance was equilibrated to the exposure temperature prior to use. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each batch of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and completely covered for 2 minutes at 20.0°C. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. At the end of the exposure time the virus-test substance mixtures were passed immediately through individual Sephadex columns and diluted serially in Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

19. MRID 489215-19 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Herpes simplex virus type 1," for Maguard 5626, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 6, 2012. Project Number A13717.

This study was conducted against Herpes simplex virus type 1 (Strain F(1); ATCC VR-733), using RK cells (rabbit kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two batches of Maguard 5626 (Batch 1621-381 and Batch 1621-382) were tested according to ATS Labs Protocol No. MC03062512.HSV1.1 (copy provided). The two batches were each prepared as a 1.9 oz/gallon dilution, defined as 1.00 mL product + 66.0 mL of 400 ppm AOAC synthetic hard water (1:67) and were used on the day of preparation. The prepared test substance was equilibrated to the exposure temperature prior to use. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each batch of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and completely covered for 2 minutes at 20.0°C. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. At the end of the exposure time the virus-test substance mixtures were passed immediately through individual Sephadex columns and diluted serially in Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

20. MRID 489215-20 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Influenza A virus," for Maguard 5626, by Dawn Pierson. Study conducted at ATS Labs. Study completion date – August 2, 2012.

Project Number A13706.

This study was conducted against Influenza A (H3N2) virus, (Strain Hong Kong, ATCC VR-544), using RMK cells (Rhesus monkey kidney cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two batches of Maguard 5626 (Batch 1621-381 and Batch 1621-382) were tested according to ATS Labs Protocol No. MC03062512.FLUA.1 (copy provided). The two batches were each prepared as a 1.9 oz/gallon dilution, defined as 1.00 mL product + 66.0 mL of 400 ppm AOAC synthetic hard water (1:67) and were used on the day of preparation. The prepared test substance was equilibrated to the exposure temperature prior to use. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate 100 x 15 mm sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each batch of test substance, one dried virus film was individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and completely covered for 2 minutes at 20.0°C. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents. At the end of the exposure time the virus-test substance mixtures were passed immediately through individual Sephadex columns and diluted serially in Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

21. MRID 489215-21 "AOAC Use Dilution Method, Test Organism: *Klebsiella pneumoniae* (ATCC 4352)", for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13764.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested ATS Laboratory, Protocol No. MC03062512.UD.10 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz./gallon dilution, using 2.00 mL of test substance + 132.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. The contaminated and dried carriers were individually transferred to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were

examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

22. MRID 489215-22 "AOAC Use Dilution Method, Test Organism: *Klebsiella pneumoniae* (ATCC 4352)", for Maguard 5626, by Anne Stemper, B.S. Study conducted at ATS Labs. Study completion date – Aug 13, 2012. Project Number A13767.

This study was conducted against *Klebsiella pneumoniae* (ATCC 4352). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Laboratory, Protocol No. MC03062512/UD/26 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz./gallon dilution, using 2.00 mL of test substance + 132.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. The contaminated and dried carriers were individually transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred by hook needle at identical staggered intervals to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

23. MRID 489215-23 "AOAC Use Dilution Method, Test Organism: *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705)", for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – July 31, 2012. Project Number A13719.

This study was conducted against *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested ATS Laboratory Protocol No. MC03062512.UD.11 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz/gallon dilution, using 2.00 mL of test substance + 132.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 40%

relative humidity. The contaminated and dried carriers were individually transferred to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) was verified on a representative culture. The laboratory performed a Modified Hodge Test to confirm that the test organism produces a carbapenemase and is, therefore, carbapenem resistant. *Klebsiella pneumoniae* (ATCC BAA-1705) was the positive control organism. *Klebsiella pneumoniae* (ATCC BAA-1706) was the negative control organism. By inactivating the effect of meropenem and allowing growth of *Escherichia coli* (ATCC 25922), the presence of carbapenemase was demonstrated. Thus, antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) to carbapenem was confirmed. See page 16 and Table 5 of the laboratory report.

24. MRID 489215-24 "AOAC Use Dilution Method, Test Organism: *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705)", for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 2, 2012. Project Number A13714.

This study was conducted against *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.UD.27 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz./gallon dilution, using 2.00 mL of test substance + 132.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. The contaminated and dried carriers were individually transferred to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

Note: Antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) was verified on a representative culture. The laboratory performed a Modified Hodge Test to confirm that the test organism produces a carbapenemase and is, therefore,

carbapenem resistant. *Klebsiella pneumoniae* (ATCC BAA-1705) was the positive control organism. *Klebsiella pneumoniae* (ATCC BAA-1706) was the negative control organism. By inactivating the effect of meropenem and allowing growth of *Escherichia coli* (ATCC 25922), the presence of carbapenemase was demonstrated. Thus, antibiotic resistance of *Klebsiella pneumoniae* Carbapenem Resistant (ATCC BAA-1705) to carbapenem was confirmed. See page 16 and Table 5 of the laboratory report.

25. MRID 489215-25 "AOAC Use Dilution Method, Test Organism: *Proteus mirabilis* (ATCC 9240)", for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13765.

This study was conducted against *Proteus mirabilis* (ATCC 9240). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested ATS Laboratory Protocol No. MC03062512.UD.13 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz./gallon dilution, using 4.00 mL of test substance + 264.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. The contaminated and dried carriers were individually transferred to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

26. MRID 489215-26 "AOAC Use Dilution Method, Test Organism: *Proteus mirabilis* (ATCC 9240)", for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13768.

This study was conducted against *Proteus mirabilis* (ATCC 9240). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested ATS Laboratory Protocol No. MC03062512.UD.29 (copy provided). The object of this study was to determine the effectiveness of the Sponsor's product as a disinfectant for hard surfaces following the AOAC Use-Dilution Method. This method is in compliance with the requirements of the U.S. Environmental Protection Agency (EPA). The test substance was prepared as a 1.9 oz./gallon dilution, using 4.00 mL of test substance + 264.0 mL of 400 ppm AOAC synthetic hard water (1:67) and was used within 3 hours of preparation. Ten (10.0) mL aliquots of the test substance at the concentration under test were transferred to sterile 25 x 150 mm tubes, placed in a 20±1°C water bath and allowed to equilibrate for ≥10 minutes prior to testing. A 1.00 mL aliquot of fetal bovine serum was added to 19.0 mL of prepared culture to achieve a 5% fetal bovine serum organic soil load. The final prepared test culture was transferred to the penicylinder carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one

carrier per one mL of culture. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. The contaminated and dried carriers were individually transferred to individual tubes containing 10.0 mL of the prepared test substance at the requested use dilution and were exposed for 2 minutes at 20±1°C (20.0°C). Following the exposure period, the individual carriers were transferred to 10 mL of Letheen Broth with 0.1% sodium thiosulfate. All subcultures and control plates were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, neutralization confirmation, and antibiotic resistance.

27. MRID 489215-27 "AOAC Use-Dilution Method, Test Organism: *Pseudomonas aeruginosa* (ATCC 15442)" for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – June 20, 2012. Project Number A13375.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot 1621-381) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03051612.UD.1 (copy provided). A use solution was prepared by adding 20.0 mL of the product and 1320 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

28. MRID 489215-28 "AOAC Use-Dilution Method, Test Organism: *Pseudomonas aeruginosa* (ATCC 15442)" for Maguard 5626, by Becky Lien. Study conducted at ATS Labs. Study completion date – June 15, 2012. Project Number A13377.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot 1621-382) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03051612.UD.3 (copy provided). A use solution was prepared by adding 20.0 mL of the product and 1320 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible

growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

29. MRID 489215-29 "AOAC Use-Dilution Method, Test Organism: *Pseudomonas aeruginosa* (ATCC 15442)" for Maguard 5626 by Jill Ruhme. Study conducted at ATS Labs. Study completion date – May 29, 2012. Project Number A13144.

This study was conducted against *Pseudomonas aeruginosa* (ATCC 15442). One lot (Lot ZC) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03041812.UD (copy provided). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 12.0 mL of the product and 792 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). A culture of the challenge microorganism was prepared in accordance with the published AOAC methods. Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 51% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 1 day at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

30. MRID 489215-30 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rhinovirus type 37 for Maguard 5626, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13722.

This study was conducted against Rhinovirus type 37 (Strain 151-1; ATCC VR-1147), using MRC-5 cells (human embryonic lung from ATCC (ATCC CCL-171) as the host system. Two lots (Lots 1621-381 and 1621-382) of the product, Maguard 5626, were tested according to ATS Labs Protocol No. MC03062512.R37.1 (copy provided). A use solution was prepared by adding 1 mL of the product and 66 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes (100 x 15 mm). The virus films were air-dried for 20 minutes at 15.5°C at 55% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 2 minutes at 20.0±1°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in serum-free Minimum Essential Medium with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

31. MRID 489215-31 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Rotavirus" for Maguard 5626, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 14, 2012. Project Number A13723.

This study was conducted against rotavirus (Strain WA; obtained from the University of Ottawa, Ontario, Canada), using MA-104 cells (Rhesus monkey kidney cells; obtained from Diagnostics Hybrids, Inc., Athens, OH; propagated in-house) as the host system. Two lots (Lots 1621-381 and 1621-382) of the product, Maguard 5626, were tested according to ATS Labs Protocol No. MC03062512.ROT.1 (copy provided). A use solution was prepared by adding 1 mL of the product and 66 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes (100 x 15 mm). The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 2 minutes at 20.0±1°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in serum-free Minimum Essential Medium with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. MA-104 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The inoculum was allowed to adsorb for 80 minutes at 36-38°C in a humidified atmosphere of 5-7% CO_2. Post-adsorption, the cultures were re-fed. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

32. MRID 489215-32 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Respiratory Syncytial (RSV) Virus for Maguard 5626, by Dawn Pierson. Study conducted at ATS Labs. Study completion date – July 31, 2012. Project Number A13698.

This study was conducted against the Long strain of Respiratory Syncytial (RSV) Virus (ATCC VR-26), using Hep-2 (human larynx carcinoma) cells as the host system. Two lots (Lots 1621-381 and 1621-382) of the product, Maguard 5626, were tested according to ATS Labs Protocol No. MC03062512.RSV.1 (copy provided). A use solution was prepared by adding 1 mL of the product and 66 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes (100 x 15 mm). The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 2 minutes at 20.0±1°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in serum-free Minimum Essential Medium with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 10

days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

33. MRID 489215-33 "AOAC Use-Dilution Method, Test Organism: *Salmonella enterica* (ATCC 10708)" for Maguard 5626, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – August 14, 2012. Project Number A13797.

This study was conducted against *Salmonella enterica* (ATCC 10708). One lot (1621-381) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.UD.31 (copy provided). A use solution was prepared by adding 10 mL of the product and 660 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

34. MRID 489215-34 "AOAC Use-Dilution Method, Test Organism: *Salmonella enterica* (ATCC 10708)" for Maguard 5626, by Jill Ruhme. Study conducted at ATS Labs. Study completion date – August 16, 2012. Project Number A13799.

This study was conducted against *Salmonella enterica* (ATCC 10708). One lot (Lot ZC) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.UD.33 (copy provided). The product lot tested was at least 60 days old at the time of testing. A use solution was prepared by adding 10 mL of the product and 660 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Sixty (60) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 48-54 hour old suspension of test organism, at a ratio of 1 carrier per 1 mL broth. The carriers were dried for 38 minutes at 35-37°C at 55.2% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 19.0°C. Following exposure, individual carriers were transferred to 10 mL of Letheen Broth with 0.07% Lecithin and 0.5% Tween 80. All subcultures were incubated for 48±2 hours at 35-37°C. The subcultures were stored for 2 days at 2-8°C prior to examination. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

35. MRID 489215-35: "AOAC Use-Dilution Method, Test Organism: *Staphylococcus aureus* (ATCC 6538) for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – June 28, 2012. Project Number A13376.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03051612.UD.2 (copy provided). The product was prepared using 10 mL of the test substance per 660 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine

serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 60 carriers immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 40% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: In testing performed on 6/8/12, neutralization confirmation controls failed to give the desired results. Testing was repeated on 6/21/12 which gave valid results for the neutralization confirmation controls.

36. MRID 489215-36: "AOAC Use-Dilution Method, Test Organism: *Staphylococcus aureus* (ATCC 6538) for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – August 2, 2012. Project Number A13736.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062812.UD (copy provided). The product was prepared using 11 mL of the test substance per 726 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 60 carriers immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 50% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

37. MRID 489215-37: "AOAC Use-Dilution Method, Test Organism: *Staphylococcus aureus* (ATCC 6538) for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – July 11, 2012. Project Number A13608.

This study was conducted against *Staphylococcus aureus* (ATCC 6538). One lot (Lot ZC, ≥60 days old) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062012.UD (copy provided). The product was prepared using 10 mL of the test substance per 660 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 60 carriers immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 54.6% relative humidity, for 40 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity,

sterility, viability, neutralization confirmation, and carrier population. A deviation from protocol occurred when it was discovered that the lab inadvertently failed to place a carrier in the carrier sterility tube. However, since there was no growth in any of the test carriers, this omission did not affect interpretation of the results.

38. MRID 489215-38 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Vaccinia Virus for Maguard 5626, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – August 13, 2012. Project Number A13715.

This study was conducted against the WR strain of Vaccinia virus (ATCC VR-119), using Vero cells (ATCC CCL-81) as the host system. Two lots (Lots 1621-381 and 1621-382) of the product, Maguard 5626, were tested according to ATS Labs Protocol No. MC03062512.VAC.1 (copy provided). A use solution was prepared by adding 1 mL of the product and 66 mL of 400 ppm AOAC synthetic hard water (1.9 oz/gal defined as 1 part test substance + 66 parts of 400 ppm AOAC synthetic hard water – 1:67). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes (100 x 15 mm). The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were exposed to 2.00 mL of the use solution for 2 minutes at 20.0±1°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in serum-free Minimum Essential Medium with 0.5 µg/mL trypsin, 2.0 mM L-glutamine, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Vero cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

39. MRID 489215-39: "AOAC Use-Dilution Method, Test Organism: Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836)"for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13763.

This study was conducted against Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.4 (copy provided). The product was prepared using 4 mL of the test substance per 264.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 50% relative humidity, for 38 minutes at 25-30°C or 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C.

Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. A control agar was prepared using *Staphylococcus aureus* (ATCC 29213) as a control organism. A Vancomycin Etest strip was placed on each plate. The plates were incubated and, following incubation, the Minimum Inhibitory Concentration (MIC) was read. The measurement confirmed intermediate antibiotic resistance of Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836) to Vancomycin. See Table 5 on page 17 of the laboratory report.

40. MRID 489215-40: "AOAC Use-Dilution Method, Test Organism: Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836)"for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13766.

This study was conducted against Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.19 (copy provided). The product was prepared using 4 mL of the test substance per 264.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal– 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 50% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836) was verified on a representative culture. An individual Mueller Hinton agar plate was streaked with the prepared culture. A control agar was prepared using *Staphylococcus aureus* (ATCC 29213) as a control organism. A Vancomycin Etest strip was placed on each plate. The plates were incubated and, following incubation, the Minimum Inhibitory Concentration (MIC) was read. The measurement confirmed intermediate antibiotic resistance of Vancomycin Intermediate Resistant *Staphylococcus aureus* – VISA (HIP 5836) to Vancomycin. See Table 5 on page 18 of the laboratory report.

41. MRID 489215-41: "AOAC Use-Dilution Method, Test Organism: Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)" for Maguard 5626 (10324-214) by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 24, 2012. Project Number A13705.

This study was conducted against Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575). One lot (Lot No. 1621-381) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.5 (copy provided). The product was prepared using 2 mL of

the test substance per 132.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 65% relative humidity, for 38 minutes at 25-30°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575) to vancomycin. See Table 5 on page 18 of the laboratory report.
Note: The quality control [*Staphyloccous aureus* (ATCC 25923)] test for antimicrobial susceptibility did not give acceptable results, thus the Kirby Bauer Susceptibility Method for the test organism and the control were repeated and gave acceptable results.

42. MRID 489215-42. "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for Norovirus" for Maguard 5626, by Mary Miller. Study conducted at ATS Labs. Study completion date – September 10, 2012. Project Number A13958.

This study was conducted against Feline calicivirus (Strain F-9; ATCC VR-782), using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. One batch (1621-395) of the product, Maguard 5626, was tested according to ATS Labs Protocol No. MC03082012.FCAL.2 (copy provided). The test substance was prepared by diluting 1 mL product with 31 mL 400 ppm hard water (4 oz/gallon – 1:32). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0ºC at 50% relative humidity. Two replicates were tested. The dried films were exposed (completely covered) to 2.0 mL of the use dilution of the test substance at 20±1ºC and held covered for 2 minutes. Just prior to the end of the exposure time, each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially using Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

43. MRID 489215-43. "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Feline Calicivirus as a Surrogate Virus for

Norovirus" for Maguard 5626, by Mary Miller. Study conducted at ATS Labs. Study completion date – September 7, 2012. Project Number A13956.

This study was conducted against Feline calicivirus (Strain F-9; ATCC VR-782), using CRFK cells (Crandel Reese feline kidney cells; ATCC CCL-94; propagated in-house) as the host system. One batch (1621-394) of the product, Maguard 5626, was tested according to ATS Labs Protocol No. MC03082012.FCAL.1 (copy provided). The test substance was prepared by diluting 1 mL product with 31mL 400 ppm hard water (4 oz/gallon). The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0ºC at 50% relative humidity. Two replicates were tested. The dried films were exposed (completely covered) to 2.0 mL of the use dilution of the test substance at 20±1ºC and held covered for 2 minutes. Just prior to the end of the exposure time, each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially using Minimum Essential Medium with 5% (v/v) heat inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 31-35°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (i.e., small, rounding of the cells, with a slight granular look), cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

44. MRID 489215-44. "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Escherichia coli* O145:H28 (ATCC BAA-1652)," for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date September 11, 2012. Project Number A13944.

This study was conducted against *Escherichia coli* O145:H28 (ATCC BAA-1652). Two lots (Lots 1621-394 and 1621-395; 2 runs of each) of the product were tested using ATS Laboratory Protocol No. MC03081712.GDST.1 (copy provided). Use solutions were prepared by adding 1.00 mL of the product and 767 mL of 500 ppm AOAC synthetic hard water (1:768). The culture suspension was adjusted to target a 1 x 10^{10} CFU/mL culture suspension. The absorbance value of the culture suspension was measured (at 620 nm) using a spectrophotometer; the absorbance value was determined to be 1.864. Use solutions were not tested in the presence of a 5% organic soil load. A 99.0-mL aliquot of each use solution was transferred to duplicate 250-300 mL Erlenmeyer flasks and placed in a water bath at 25.0°C. A 1 mL bacterial suspension was added to each flask. Following a 30 second exposure, 1-mL aliquots of the bacterium-product mixture were transferred to 9 mL of Letheen Broth with 0.1% sodium thiosulfate. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in Tryptone glucose extract agar. All plates were incubated for 48±4 hours at 35-37°C. Following incubation the colonies were visually examined for growth. Controls included those for numbers count, purity, sterility, viability, and neutralization confirmation.

45. MRID 489215-45 "Fungicidal Use-Dilution Method, Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)" for Maguard 5636, by Joshua Luedtke. Study conducted at ATS Labs. Study completion date – September 13, 2012. Project Number A13889.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). One lot (1621-394) of the product was tested using ATS Laboratory Protocol No. MC030801312.FUD (copy provided). Use solutions were prepared by adding 4.00 mL of the product and 124 mL of 400 ppm AOAC synthetic hard water (1:32; titrated at 400 ppm). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 10-day old suspension of test organism, at a ratio of 1 carrier per 1 mL suspension. The carriers were dried for 38 minutes at 35-37°C at 50% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth with 0.1% sodium thiosulfate to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 at least 30 minutes following the first transfer. All subcultures were incubated for 10 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

46. MRID 489215-46 "Fungicidal Use-Dilution Method, Test Organism: *Trichophyton mentagrophytes* (ATCC 9533)" for Maguard 5626, by Anne Stember. Study conducted at ATS Labs. Study completion date – August 10, 2012. Project Number A13703.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). One lot (1621-381) of the product was tested using ATS Laboratory Protocol No. MC03062512.FUD.1 (copy provided). Use solutions were prepared by adding 4.00 mL of the product and 124 mL of 400 ppm AOAC synthetic hard water (1:32; titrated at 400 ppm). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed for 15±2 minutes in a 10-day old suspension of test organism, at a ratio of 1 carrier per 1 mL suspension. The carriers were dried for 38 minutes at 35-37°C at 40% relative humidity. Each carrier was placed in 10.0 mL of the use solution for 2 minutes at 20±1°C. Following exposure, individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth with 0.1% sodium thiosulfate to neutralize. Carriers were transferred from primary subculture tubes into individual secondary subculture tubes containing 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 at least 30 minutes following the first transfer. All subcultures were incubated for 10 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

47. MRID 489215-47*: "*AOAC Use-Dilution Method, Test Organism*: Vancomycin Resistant *Enterococcus faecalis* – VRE (ATCC 51575)" for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 24, 2012. Project Number A13708.

This study was conducted against Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575). One lot (Lot No. 1621-382) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03062512.UD.20 (copy provided). The product was prepared using 4 mL of the test substance per 264.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 65% relative humidity, for 38 minutes at 25-30°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in

contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

Note: Antibiotic resistance of Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575) was verified on a representative culture. The laboratory performed a Kirby Bauer Susceptibility assay. *Staphylococcus aureus* (ATCC 25923) was the control organism. The measured zone of inhibition confirmed antibiotic resistance of Vancomycin Resistant Enterococcus *faecalis* – VRE (ATCC 51575) to vancomycin. See Table 5 on page 18 of the laboratory report.
Note: The quality control [*Staphyloccous aureus* (ATCC 25923)] test for antimicrobial susceptibility did not give acceptable results, thus the Kirby Bauer Susceptibility Method for the test organism and the control were repeated and gave acceptable results.

48. MRID 489215-48 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Human Immunodeficiency Virus Type 1," for Maguard 5626, by Shanen Conway. Study conducted at ATS Labs. Study completion date – August 27, 2012. Project Number A13818.

This study was conducted against Human immunodeficiency virus type 1 (Strain HTLV-III_B; obtained from Advanced Biotechnologies, Inc., Columbia, MD), using MT-2 cells (human T-cell leukemia cells; obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH; maintained in-house) as the host system. The test product was diluted (1:67) in 400 ppm hard water. Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested according to ATS Labs Protocol No. MC03062512.HIV.1 (copy provided). The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 21.0°C. For each lot of product, one dried virus film was individually covered for 2.00 mL of the test product dilution and held covered for 2 minutes at 21.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially in RPMI-1640 with 15% (v/v) heat inactivated fetal bovine serum, 2.0 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.2 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

49. MRID 489215-49 "Fungicidal Use-Dilution, Test Organism: *Candida albicans* (ATCC 10231)" for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – August 24, 2012. Project Number A13709.

This study was conducted against *Candida albicans* (ATCC 10231). One lot (1621-382) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.FUD.3 (copy provided). A dilution of one part test substance and 66 parts 400 ppm hard water (1:67) was used. Fetal bovine serum was added to to yield a 5% organic soil load. The culture was transferred to 10 carriers which were immersed for 15±2 minutes in a

prepared suspension at a ratio of one carrier per mL of culture. The carriers were dried for 38 minutes at 25-30°C at 65% humidity. The contaminated and dried carriers were exposed to the test substance for 2 minutes at 20±1°C. Following the exposure period, the individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth and 0.1% sodium thiosulfate to neutralize. Carriers were then transferred from the primary subcultures into individual secondary subcultures containing 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 for at least 30 minutes following the first transfer. Neutralization subcultures were incubated for 3 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

Note: The neutralization test had to be repeated because in the initial test, the primary subcultures were inadvertently not incubated. The results of the repeated assay were considered valid. Results of the initial test are included in the study report as Attachment 1.

50. MRID 489215-50 "Fungicidal Use-Dilution, Test Organism: *Candida albicans* (ATCC 10231)" for Maguard 5626, by Nicole Albert. Study conducted at ATS Labs. Study completion date – July 31, 2012. Project Number A13734.

This study was conducted against *Candida albicans* (ATCC 10231). One lot (1621-381) of the product, Maguard 5626, was tested using ATS Laboratory Protocol No. MC03062512.FUD.2 (copy provided). A dilution of one part test substance and 66 parts 400 ppm hard water (1:67) was used. Fetal bovine serum was added to to yield a 5% organic soil load. The culture was transferred to 10 carriers which were immersed for 15±2 minutes in a prepared suspension at a ratio of one carrier per mL of culture. The carriers were dried for 38 minutes at 25-30°C at 66% humidity. The contaminated and dried carriers were exposed to the test substance for 2 minutes at 20±1°C. Following the exposure period, the individual carriers were transferred to 10 mL of Sabouraud Dextrose Broth and 0.1% sodium thiosulfate to neutralize. Carriers were then transferred from the primary subcultures into individual secondary subcultures containing 10 mL of Sabouraud Dextrose Broth with 0.07% Lecithin and 0.5% Tween 80 for at least 30 minutes following the first transfer. Neutralization subcultures were incubated for 3 days at 25-30°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included those for carrier population, purity, sterility, viability, and neutralization confirmation.

51. MRID 489215-51: "AOAC Use-Dilution Method, Test Organism: *Streptococcus pneumoniae* (ATCC 6305)" for Maguard 5626, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – August 29, 2012. Project Number A13808.

This study was conducted against *Streptococcus pneumoniae* (ATCC 6305). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03062512.UD.6 (copy provided). The product was prepared using 10 mL of the test substance and 660 mL AOAC synthetic 400 ppm hard water (1.9 fl oz/gal; 1:67)). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to the penicylinders and 10 carriers per lot were immersed for 15±2 minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 67% relative humidity, for 38 minutes at 25-30°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20°C. Individual carriers were transferred to primary subculture tubes containing 10 mL of Brain Heart Infusion and 0.1% Sodium Thiosulfate and then to secondary tubes containing 10 mL of Brain Heart Infusion ≥30 minutes

after subculture of the first carriers. All subcultures were incubated for 48±2 hours at 35-37°C in CO_2. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

52. MRID 489215-52: "AOAC Use-Dilution Method, Test Organisms: Community Acquired Methicillin Resistant *Staphylococcus aureus* [CA-MRSA Genotype USA 300 (NRS 384)]" for Maguard 5626 (10324-214) by Matthew Sathe. Study conducted at ATS Labs. Study completion date – August 29, 2012. Project Number A13807.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* - MRSA Genotype USA 300 (NRS 384). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03062512.UD.2 (copy provided). The product was prepared using 10 mL of the test substance per 660 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 41% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

53. MRID 489215-53: "AOAC Use-Dilution Method, Test Organism: *Streptococcus pyogenes* (ATCC 19615)"for Maguard 5626, by Matthew Sathe. Study conducted at ATS Labs. Study completion date – August 29, 2012. Project Number A13809.

This study was conducted against *Streptococcus pyogenes* (ATCC 19615). Two lots (Lot Nos. 1621-381 and 1621-382) of the product, Maguard 5626, were tested using ATS Labs protocol # MC03062512.UD.7 (copy provided). The product was prepared using 10 mL of the test substance and 660 mL AOAC synthetic 400 ppm hard water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to the penicylinders and 10 carriers per lot were immersed for 15±2 minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 67% relative humidity, for 38 minutes at 25-30°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to primary subculture tubes containing 10 mL of Brain Heart Infusion containing 0.1% Sodium Thiosulfate and then to secondary tubes containing 10 mL of Brain Heart Infusion ≥30 minutes after subculture of the first carriers. All subcultures were incubated for 48±2 hours at 35-37°C in CO_2. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

54. MRID 489215-54: "AOAC Use-Dilution Method, Test Organism: Methicillin Resistant *Staphylococcus aureus* – MRSA (ATCC 33592)" for Maguard 5626, by

Anne Stemper. Study conducted at ATS Labs. Study completion date – September 19, 2012. Project Number A13968.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592). One lot (Lot No. 1621-394) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03082212.UD.1 (copy provided). The product was prepared using 2 mL of the test substance per 132.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal– 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 54.5% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

55. MRID 489215-55: "AOAC Use-Dilution Method, Test Organism: Methicillin Resistant *Staphylococcus aureus* – MRSA (ATCC 33592)" for Maguard 5626, by Anne Stemper. Study conducted at ATS Labs. Study completion date – September 21, 2012. Project Number A13969.

This study was conducted against Methicillin Resistant *Staphylococcus aureus* - MRSA (ATCC 33592). One lot (Lot No. 1621-395) of the product, Maguard 5626, was tested using ATS Labs protocol # MC03082212.UD.2 (copy provided). The product was prepared using 2 mL of the test substance per 132.0 mL of 400 ppm AOAC synthetic water (1.9 fl oz/gal – 1:67). Fetal bovine serum was added to the culture to achieve a 5% organic soil load. The culture was then transferred to penicylinders and 10 carriers were immersed for 15± minutes at a ratio of one carrier per one mL of culture. The carriers were dried at 54.5% relative humidity, for 38 minutes at 35-37°C. Each carrier was transferred by hook needle at staggered intervals to individual tubes containing 10.0 mL of the test substance at the desired dilution. Each carrier remained in contact with the product for 2 minutes at 20±1°C. Individual carriers were transferred to 10 mL of Letheen Broth containing 0.1% Sodium Thiosulfate for neutralization and subculturing. All subcultures were incubated for 48±2 hours at 35-37°C. Following incubation, the subcultures were visually examined for the presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

56. MRID 489215-56. "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus" for Maguard 5626, by Mary Miller. Study conducted at ATS Labs. Study completion date – September 26, 2012. Project Number A14009.

This study was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from National Veterinary Services Laboratories, Ames IA), using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. One lot (Lot No. 1621-394) of the product was tested according to ATS Protocol No. MC03090712.BVD.1 (copy provided). The test substance was prepared by diluting 1 mL product with 31 mL 400 ppm hard water (4 oz/gallon – 1:32). The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of

virus inoculum uniformly over bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Two replicates were tested. The dried films were exposed (completely covered) to 2.0 mL of the use dilution of the test substance at 20±1°C and held covered for 5 minutes. Following exposure, each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially using Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (CPE; i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

57. MRID 489215-57. "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Bovine Viral Diarrhea Virus as a Surrogate Virus for Human Hepatitis C Virus" for Maguard 5626, by Mary Miller. Study conducted at ATS Labs. Study completion date – September 26, 2012. Project Number A14010.

This study was conducted against Bovine viral diarrhea virus (Strain Oregon C24v-genotype 1; obtained from National Veterinary Services Laboratories, Ames IA), using bovine turbinate cells (BT cells; ATCC CRL-1390; propagated in-house) as the host system. One lot (Lot No. 1621-395) of the product was tested according to ATS Protocol No. MC03090712.BVD.2 (copy provided). The test substance was prepared by diluting 1 mL product with 31 mL 400 ppm hard water (4 oz/gallon – 1:32)). The stock virus culture contained 5% horse serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 40% relative humidity. Two replicates were tested. The dried films were exposed (completely covered) to 2.0 mL of the use dilution of the test substance at 20±1°C and held covered for 5 minutes. Following exposure, each plate was scraped with a cell scraper to re-suspend the contents. The virus-disinfectant mixtures were passed immediately through individual Sephadex columns, and diluted serially using Minimum Essential Medium with 5% (v/v) non-heat inactivated horse serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. BT cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of selected dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The cultures were scored periodically for 7 days for the presence or absence of cytopathic effects (CPE; i.e., rounded, dark, with a granular appearance), cytotoxicity, and viability. On the final day of incubation, infectious virus was assayed by a direct immunofluorescence assay to verify the CPE reading. Controls included those for input virus count, dried virus count, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

58. MRID 489215-58: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Maguard 5626, Batch 1621-394, by Shanen Conway. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14025.

This study was conducted against Duck Hepatitis B Virus (DHBV; Strain 10/29/11) in the serum of congenitally infected ducklings obtained from Hepadnavirus Testing, Inc., Palo Alto, CA. One lot (Batch 1621-394) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.DHBV.1 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 1 parts test substance + 31 parts of 400 ppm AOAC Synthetic Hard Water. The whole duck serum containing the virus served as the organic soil load. Purified hepatocytes obtained from ducklings screened for Hepatitis B infectivity were used as the indicator cell cultures. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of four separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). Two dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for five minutes at room temperature (20±1°C). The virus films were completely covered with the use solution. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixtures were passed through individual Sephadex columns to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Primary duck hepatocytes from uninfected ducklings were used as the indicator cell line in the infectivity assays. Cells in multi-well culture dishes containing 1.0 mL of culture medium were inoculated in quadruplicate with 250 µL of the dilutions prepared from the test and control groups. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2 after which 1.0 mL of test medium was added to each well. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for nine days. On the final day of incubation the cultures were microscopically scored for cytotoxicity and an indirect immunofluorescence assay was performed using a monoclonal antibody specific for the protein envelope of the DHBV. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The log10 reduction in infectivity was calculated using a Most Probable Number (MPN) statistical method. Control groups included a negative control, input (non-dried) virus control, dried virus control (in duplicate), cytotoxicity control, and neutralization control.

59. MRID 489215-59: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B Virus as a Surrogate Virus for Human Hepatitis B Virus" for Maguard 5626, Batch #1621-395, by Shanen Conway. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14026.

This study was conducted against Duck Hepatitis B Virus (DHBV; Strain 10/29/11) in the serum of congenitally infected ducklings obtained from Hepadnavirus Testing, Inc., Palo Alto, CA. One lot (Batch 1621-395) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.DHBV.2 (copy provided). The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 1 part test substance + 31 parts of 400 ppm AOAC Synthetic Hard Water. The whole duck serum containing the virus served as the organic soil load. Purified hepatocytes obtained from ducklings screened for Hepatitis B infectivity were used as the indicator cell cultures. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of four separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). Two dried virus films were individually exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for five minutes at room temperature (20±1°C). The virus films were completely covered with the use solution. Just prior to the end of the exposure time, the plates were scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixtures were passed through

individual Sephadex columns to detoxify the mixtures. The filtrates (10^{-1} dilution) were then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Primary duck hepatocytes from uninfected ducklings were used as the indicator cell line in the infectivity assays. Cells in multi-well culture dishes containing 1.0 mL of culture medium were inoculated in quadruplicate with 250 µL of the dilutions prepared from the test and control groups. The inoculum was allowed to adsorb overnight at 36-38°C in a humidified atmosphere of 5-7% CO_2 after which 1.0 mL of medium was added to each well. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2 for nine days. On the final day of incubation the cultures were microscopically scored for cytotoxicity and an in direct immunofluorescence assay was performed using a monoclonal antibody specific for the protein envelope of the DHBV. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. The log10 reduction in infectivity was calculated using a Most Probable Number (MPN) statistical method. Control groups included a negative control, input (nondried) virus control, dried virus control (in duplicate), cytotoxicity control, and neutralization control.

60. MRID 489215-60: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Canine Parvovirus" for Maguard 5626, Batch #1621-394, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14031.

This study was conducted against Canine Parvovirus (Cornell-780916-80 strain) obtained from the American Type Culture Collection. One lot (Batch 1621-394) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.CPV.1 (copy provided). The stock virus culture contained a 5% organic soil load (fetal bovine serum). Cultures of A-72 cells (canine tumor, CRL-1542) obtained from the American Type Culture Collection were used as the infectivity indicators. The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 4 parts test substance + 124 parts of 400 ppm AOAC Synthetic Hard Water. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of two separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). One dried virus film was exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for five minutes at 20±1°C. The virus film was completely covered with the use solution. Just prior to the end of the exposure time, the plate was scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixture was passed through a Sephadex column to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. A-72 canine tumor cells, which exhibit cytopathic effects (CPE) in the presence of canine Parvovirus were used as the infectivity indicators. Cells in multi-well culture dishes containing culture medium were inoculated in quadruplicate with 100 µL of the dilutions prepared in the test and control groups. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, and were scored periodically for seven days for the presence or absence of CPE, cytotoxicity and for viability. On the final day of incubation, a hemagglutination assay was performed on the cultures using swine red blood cells at 2-8°C. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Control groups included an input (nondried) virus control, dried virus control, cytotoxicity control, and neutralization control.

Note: Protocol amendment reported in the study (change in ATCC number for A-72 canine tumor cell culture) was reviewed.

61. MRID 489215-61: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Canine Parvovirus" for Maguard 5626, Batch

#1621-395, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14032.

This study was conducted against Canine Parvovirus (Cornell-780916-80 strain) obtained from the American Type Culture Collection. One lot (Batch 1621-395) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.CPV.2 (copy provided). The stock virus culture contained a 5% organic soil load (fetal bovine serum). Cultures of A-72 cells (canine tumor, CRL-1542) obtained from the American Type Culture Collection were used as the infectivity indicators. The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 4 parts test substance + 124 parts of 400 ppm AOAC Synthetic Hard Water. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of two separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). One dried virus film was exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for five minutes at 20±1°C. The virus film was completely covered with the use solution. Just prior to the end of the exposure time, the plate was scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixture was passed through a Sephadex column to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. The A-72 canine tumor cell line, which exhibits cytopathic effects (CPE) in the presence of Canine Parvovirus was used as the infectivity indicator. Cells in multi-well culture dishes containing culture medium were inoculated in quadruplicate with 100 µL of the dilutions prepared in the test and control groups. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, and were scored periodically for seven days for the presence or absence of CPE, cytotoxicity and for viability. On the final day of incubation, a hemagglutination assay was performed on the cultures using swine red blood cells at 2-8°C. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Control groups included an input (nondried) virus control, dried virus control, cytotoxicity control, and neutralization control.

Note: Protocol amendment reported in the study (change in ATCC number for A-72 canine tumor cell culture) was reviewed.

62. MRID 489215-62: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Murine Norovirus (MNV-1)" for Maguard 5626, Batch #1621-394, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14033.

This study was conducted against Murine Norovirus (Strain MNV-1.CW1) obtained from Washington University, St. Louis, MO. One lot (Batch 1621-394) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.MNV.1 (copy provided). The stock virus culture was adjusted to contain a 5% fetal bovine serum as the organic soil load. Cultures of RAW 264.7 cells, a continuous mouse macrophage cell line obtained from Washington University, St. Louis, MO, were used as the infectivity indicators. The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 1 parts test substance + 31 parts of 400 ppm AOAC Synthetic Hard Water. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of two separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). One dried virus film was exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for two minutes at 20±1°C. The virus film was completely covered with the use solution. Just prior to the end of the exposure time, the plate was scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test

substance mixture was passed through a Sephadex column to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Cultures of RAW 264.7 mouse macrophage cells were used as the infectivity indicators. Cells in multi-well culture dishes were inoculated in quadruplicate with 250 µL of the dilutions prepared in the test and control groups. The inoculum was allowed to adsorb for 60 min at room temperature. Following the adsorption time, the inoculum was removed and an aliquot of MNV Overlay Agarose I was inoculated into each well of the cell culture plates. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, for approximately two days. Following incubation an aliquot of MNV Overlay Agarose II containing neutral red stain was added and the culture were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, for four hours. Following incubation the cell cultures were visually observed for viral specific plaques. The cultures were microscopically examined to verify plaques or test substance cytotoxicity. Viral and cytotoxicity titers were calculated by the method of Spearman Karber. Control groups included an input (nondried) virus control, dried virus control, cytotoxicity control, and neutralization control. All cell controls were negative for test virus infectivity.

Note: Protocol amendment (changed to include a second cytotoxicity control) was reviewed.

63. MRID 489215-63: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Murine Norovirus (MNV-1)" for Maguard 5626, Batch #1621-395, by Mary J. Miller. Study conducted at ATS Labs. Study completion date – September 28, 2012. Project Number A14034.

This study was conducted against Murine Norovirus (Strain MNV-1.CW1) obtained from Washington University, St. Louis, MO. One lot (Batch 1621-395) of the product, Maguard 5626, was tested according to ATS Labs protocol # MC03090712.MNV.2 (copy provided). The stock virus culture was adjusted to contain a 5% fetal bovine serum as the organic soil load. Cultures of RAW 264.7 cells, a continuous mouse macrophage cell line obtained from Washington University, St. Louis, MO, were used as the infectivity indicators. The product was received as a concentrated solution and was diluted at a rate of 4 oz/gallon, defined as 1 parts test substance + 31 parts of 400 ppm AOAC Synthetic Hard Water. Films of virus were prepared by spreading 200 µL of virus inoculum uniformly over the bottom of two separate 100 x 15 mm sterile glass petri dishes. The virus films were air-dried at 20.0°C and 40% relative humidity until visibly dry (20 minutes). One dried virus film was exposed to a 2.00 mL aliquot of the use dilution of the test substance and held covered for two minutes at 20±1°C. The virus film was completely covered with the use solution. Just prior to the end of the exposure time, the plate was scraped with a cell scraper to re-suspend the contents, and at the end of the exposure time the virus-test substance mixture was passed through a Sephadex column to detoxify the mixture. The filtrate (10^{-1} dilution) was then titered by 10-fold serial dilution and assayed for infectivity and/or cytotoxicity. Cultures of RAW 264.7 mouse macrophage cells were used as the infectivity indicators. Cells in multi-well culture dishes were inoculated in quadruplicate with 250 µL of the dilutions prepared in the test and control groups. The inoculum was allowed to adsorb for 60 min at room temperature. Following the adsorption time, the inoculum was removed and an aliquot of MNV Overlay Agarose I was inoculated into each well of the cell culture plates. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, for approximately two days. Following incubation, an aliquot of MNV Overlay Agarose II containing neutral red stain was added and the culture were incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2, for four hours. Following incubation, the cell cultures were visually observed for viral specific plaques. The cultures were microscopically examined to verify plaques or test substance cytotoxicity. Viral and cytotoxicity titers were calculated by the method of Spearman

Karber. Control groups included an input (nondried) virus control, dried virus control, cytotoxicity control, and neutralization control. All cell controls were negative for test virus infectivity.

Note: Protocol amendment (changed to include a second cytotoxicity control) was reviewed.

64. MRID 489215-64 "Germicidal and Detergent Sanitizing Action of Disinfectants, Test Organism: *Xanthomonas axonopodis (citrus canker)* (ATCC 49118)," for Maguard 5626, Anne Stemper. Study conducted at ATS Labs. Study completion date – October 11, 2012. Project Number A14035.

This study was conducted against *Xanthomonas axonopodis (citrus canker)* (ATCC 49118). Two lots (Lot Nos. 1621-394 and 1621-395) of the product, Maguard 5626, were tested using ATS Laboratory Protocol No. MC03090612.GDST (copy provided). The use solution (a 1:384 dilution of the product) was prepared by adding 0.97 oz (Lot 1621-394) or 0.98 oz (Lot 1621-395) of the product and ~383 oz of 500 ppm AOAC synthetic hard water. Use solutions were not tested in the presence of a 5% organic soil load. 99.0-mL aliquots of the use solution were transferred to duplicate 250-300 mL Erlenmeyer flasks, placed in a water bath at 25 ± 1°C and allowed to equilibrate for ≥20 min. One-mL bacterial suspensions were added to each flask and exposed for 30 sec at 25 ± 1°C. One-mL aliquots of the bacterium-product mixture were then transferred to 9 mL of Letheen Broth containing 0.1% sodium thiosulfate and 0.01% catalase. After vortex mixing, four 1.0 mL and four 0.1 mL aliquots of the neutralized use solution were plated in tryptone glucose extract agar. All plates were incubated for 3 days at 25-30°C prior to reading. Controls included those for population, purity, neutralizer sterility, diluent sterility, PBDW (phosphate buffer dilution water) sterility, subculture agar sterility, viability, and neutralization confirmation.

V. RESULTS

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested					Carrier Population (Log_{10} CFU/ carrier)
		Lot No. 1621-381	Lot No. 1621-382	Lot No. ZC	Lot No. 1621-394	Lot No. 1621-395	
2-Minute Exposure Time at a 1:67 Dilution							
489215-01	*Acinetobacter baumannii*	0/10	---	---	---	---	6.76
489215-04	*Acinetobacter baumannii*	---	0/10	---	---	---	6.70
489215-05	*Bordetella pertussis*	0/10	0/10	---	---	---	6.83
489215-09	CA Methicillin Resistant *Staphylococcus aureus* USA 400	0/10	---	---	---	---	6.17
489215-10	CA Methicillin Resistant *Staphylococcus aureus* USA 400	---	0/10	---	---	---	6.24
489215-	*Escherichia coli*	0/10	0/10	---	---	---	7.08

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested					Carrier Population (Log_{10} CFU/ carrier)
		Lot No. 1621-381	Lot No. 1621-382	Lot No. ZC	Lot No. 1621-394	Lot No. 1621 -395	
11							
489215-15	*Escherichia coli* with extended beta-lactamase resistance	0/10	---	---	---	---	5.79
489215-16	*Escherichia coli* with extended beta-lactamase resistance	---	0/10	---	---	---	5.92
489215-21	*Klebisella pneumoniae*	0/10	---	---	---	---	6.21
489215-22	*Klebisella pneumoniae*	---	0/10	---	---	---	6.66
489215-23	*Klebisella pneumonia* Carbapenem Resistant	0/10	---	---	---	---	6.53
489215-24	*Klebisella pneumonia* Carbapenem Resistant	---	0/10	---	---	---	5.99
489215-25	*Proteus mirabilis*	0/10	---	---	---	---	6.74
489215-26	*Proteus mirabilis*	---	0/10	---	---	---	6.82
489215-27	*Pseudomonas aeruginosa*	0/60	---	---	---	---	6.85
489215-28	*Pseudomonas aeruginosa*	---	0/60	---	---	---	6.76
489215-29	*Pseudomonas aeruginosa*	---	---	0/60	---	---	6.83
489215-33	*Salmonella enterica*	0/60	---	---	---	---	6.39
489215-34	*Salmonella enterica*	---	---	1/60	---	---	6.59
489215-35	*Staphylococcus aureus*	0/60	---	---	---	---	6.06
489215-36	*Staphylococcus aureus*	---	0/60	---	---	---	6.03
489215-37	*Staphylococcus aureus*	---	---	0/60	---	---	6.38
89215-39	Vancomycin Intermediate Resistant Staphylococcus	0/10	---	---	---	---	5.71

MRID Number	Organism	No. Exhibiting Growth/Total No. Tested					Carrier Population (Log_{10} CFU/ carrier)
		Lot No. 1621-381	Lot No. 1621-382	Lot No. ZC	Lot No. 1621-394	Lot No. 1621 -395	
	aureus						
489215-40	Vancomycin Intermediate Resistant Staphylococcus *aureus*	---	0/10	---	---	---	5.82
489215-41	Vancomycin Resistant *Enterococcus faecalis*	0/10	---	---	---	---	6.12
489215-47	Vancomycin Resistant *Enterococcus faecalis*	---	0/10	---	---	---	6.15
489215-49	*Candida albicans*	---	1°=0/10 2°=0/10	---	---	---	5.99
489215-50	*Candida albicans*	1°=0/10 2°=0/10	---	---	---	---	6.81
489215-51	*Streptococcus pneumoniae*	1°=0/10 2°=0/10	1°=0/10 2°=0/10	---	---	---	5.45
489215-52	CA Methicillin Resistant *Staphylococcus aureus* USA 300	0/10	0/10	---	---	---	6.59
489215-53	*Streptococcus pyogenes*	1°=0/10 2°=0/10	1°=0/10 2°=0/10	---	---	---	6.13
489215-54	Methicillin Resistant *Staphylococcus aureus*	---	---	---	0/10	---	5.85
489215-55	Methicillin Resistant *Staphylococcus aureus*	---	---	---	---	0/10	6.17

MRID Number	Organism	No. Exhibiting Growth/ Total No. Tested		Carrier Population (Log_{10} CFU/ carrier)
		Lot 1621-381	Lot 1621-394	
2-Minute Exposure Time at a 1:32 Dilution				
489215-45	*Trichophyton mentagrophytes*	---	1°=0/10 2°=0/10	6.13
489215-46	*Trichophyton mentagrophytes*	1°=0/10 2°=0/10	---	6.03

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			CFU/carrier		
2-Minute Exposure Time at a 1:32 Dilution					
489215-06	*Clostridium difficile*	ZC	<1.00	5.01×10^6	>99.9999
489215-07	*Clostridium difficile*	1621-381	<3.09	8.13×10^6	>99.9999
489215-08	*Clostridium difficile*	1621-382	<2.51	1.10×10^7	>99.9999

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/mL)		
30-Second Exposure Time at a 1:768 Dilution					
489215-12	*Escherichia coli* O103:K.:H8	1621-381 1621-382	<1.00 <1.00	9.2×10^7 9.2×10^7	>99.999 >99.999
489215-13	*Escherichia coli* O121:K-:H10	1621-381 1621-382	$<1 \times 10^1$ 3.5×10^2	9.9×10^7 9.9×10^7	>99.999 >99.999
489215-14	*Escherichia coli* O45:K-:H-ECL	1621-381 1621-382	$<1 \times 10^1$ $<1 \times 10^1$	7.7×10^7 7.7×10^7	>99.999 >99.999
489215-17	*Escherichia coli* O26:H11	1621-381 1621-382	$<1 \times 10^1$ $<1 \times 10^1$	8.5×10^7 8.5×10^7	>99.999 >99.999
489215-44	*Escherichia coli* O145:H28	1621-394 1621-395	$<1 \times 10^1$ $<1 \times 10^1$	8.0×10^7 8.0×10^7	>99.999 >99.999

MRID Number	Organism	Lot No.	Average No. Surviving	Microbes Initially Present	Percent Reduction
			(CFU/mL)		
30-Second Exposure Time at a 1:384 Dilution					
489215-64	*Xanthomonas axonopodis* (citrus canker)	1621-394 1621-395	$<1.00 \times 10^1$ $<1.00 \times 10^1$	1.16×10^8 1.16×10^8	>99.999 >99.999

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 1621-394	Lot No. 1621-395	
5-Minute Exposure Time at a 1:32 Dilution					
489215-56	Bovine viral diarrhea (Human Hepatitis C Surrogate)	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{4.50}$ and $10^{4.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	
	Bovine viral	10^{-1} to 10^{-4}	---	Complete	$10^{5.00}$ and $10^{4.50}$

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 1621-394	Lot No. 1621-395	
489215-57	diarrhea (Human Hepatitis C Surrogate)	dilutions		inactivation	$TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	$\leq 10^{0.5}$	
489215-58	Duck Hepatitis B (Human Hepatitis B Surrogate)	10^{-1} to 10^{-4} dilutions	Complete inactivation	---	$10^{5.50}$ and $10^{5.25}$ $TCID_{50}$/0.250 mL
		$TCID_{50}$/0.25mL	$\leq 10^{0.5}$	---	
489215-59	Duck Hepatitis B (Human Hepatitis B Surrogate)	10^{-1} to 10^{-4} dilutions	---	Complete inactivation	$10^{5.50}$ and $10^{5.75}$ $TCID_{50}$/0.25mL
		$TCID_{50}$/0.25mL	---	$\leq 10^{0.5}$	
489215-60	Canine Parvovirus	10^{-1} to 10^{-7} dilutions	Complete inactivation	---	$10^{4.50}$ $TCID_{50}$/0.1 mL
		$TCID^{50}$/0.1 mL	$\leq 10^{0.5}$	---	
489215-61	Canine Parvovirus	10^{-1} to 10^{-6} dilutions	---	Complete inactivation	$10^{4.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	$\leq 10^{0.5}$	

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 1621-381	Lot No. 1621-382	
2-Minute Exposure Time at a 1:67 Dilution					
489215-18	Herpes simplex virus type 2	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-19	Herpes simplex virus type 1	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{6.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-20	Influenza A	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-30	Rhinovirus type 37	10^{-1} to 10^{-6} Dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-31	Rotavirus	10^{-1} to 10^{-7} Dilutions	Complete inactivation	Complete inactivation	$10^{6.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-32	Respiratory Syncytial (RSV)	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
489215-38	Vaccinia virus	10^{-1} to 10^{-6} dilutions	Complete inactivation	Complete inactivation	$10^{7.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
	Human immunodeficiency	10^{-1} to 10^{-8} Dilutions	Complete inactivation	Complete inactivation	$10^{5.50}$ $TCID_{50}$/0.2 mL

MRID Number	Organism	Results			Dried Virus Count
			Lot No. 1621-381	Lot No. 1621-382	
489215-48	virus type 1	$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	

MRID Number	Organism	Results					Dried Virus Count
			Lot No. 1621-381	Lot No. 1621-394	Lot No. 1621-395	Lot No. ZC	
		2-Minute Exposure Time at a 1:32 Dilution					
489215-02	Adenovirus type 5	10^{-1} to 10^{-8} dilutions	---	---	---	Complete inactivation	$10^{7.61}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	---	---	$\leq 10^{0.5}$	
489215-03	Adenovirus type 5	10^{-1} to 10^{-9} dilutions	Complete inactivation	---	---	---	$10^{7.50}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	---	---	---	
489215-42	Feline calicivirus (Surrogate for Norovirus)	10^{-1} to 10^{-7} dilutions	---	---	Complete inactivation	---	$10^{6.25}$ and $10^{6.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	---	$\leq 10^{0.5}$	---	
489215-43	Feline calicivirus (Surrogate for Norovirus)	10^{-1} to 10^{-4} dilutions	---	Complete inactivation	---	---	$10^{6.75}$ and $10^{6.75}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	---	$\leq 10^{0.5}$	---	---	
489215-62	Murine Norovirus	10^{-1} to 10^{-8} dilutions	---	Complete inactivation	---	---	$10^{5.00}$ PFU_{50}/ 0.250 mL
		PFU_{50}/0.25mL	---	$\leq 10^{0.5}$	---	---	
489215-63	Murine Norovirus	10^{-1} to 10^{-8} dilutions	---	---	Complete inactivation	---	$10^{5.00}$ PFU_{50}/ 0.250 mL
		PFU_{50}/0.25mL	---	---	$\leq 10^{0.5}$	---	

VI. CONCLUSION

1. The submitted efficacy data **support** the use of a 1:32 dilution of the product, Maguard 5626, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load (a 100% organic load against Duck hepatitis B virus) for a 5-minute contact time:

Bovine Viral Diarrhea (Surrogate for Human Hepatitis C)	MRID 489215-56 and 489215-57
Duck Hepatitis B (Surrogate for Human Hepatitis B)	MRID 489215-58 and 489215-59
Canine Parvovirus	MRID 489215-60 and 489215-61

Recoverable virus titers of at least 10^4 were achieved. Cytotoxicity (or toxicity) was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

2. The submitted efficacy data **support** the use of a 1:67 dilution of the product, Maguard 5626, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 2-minute contact time:

Herpes simplex virus type 2	MRID 489215-18
Herpes simplex virus type 1	MRID 489215-19
Influenza A	MRID 489215-20
Rhinovirus type 37	MRID 489215-30
Rotavirus	MRID 489215-31
Respiratory Syncytial (RSV)	MRID 489215-32
Vaccinia virus	MRID 489215-38
Human immunodeficiency virus type 1	MRID 489215-48

Recoverable virus titers of at least 10^4 were achieved. In studies against Human immunodeficiency virus type 1, cytotoxicity was observed in the 10^{-1} dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level. In studies against all other viruses, cytotoxicity was not observed. Complete inactivation (no growth) was indicated in all dilutions tested.

3. The submitted efficacy data **support** the use of a 1:32 dilution of the product, Maguard 5626, as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 2-minute contact time:

Adenovirus type 5	MRID 489215-02 and 489215-03
Norovirus	MRID 489215-42 and 489215-43
Murine Norovirus	MRID 489215-62 and 489215-63

Recoverable virus titers of at least 10^4 were achieved. No cytotoxicity was observed. Complete inactivation (no growth) was indicated in all dilutions tested.

4. The submitted efficacy data **support** the use of a 1:67 dilution of the product, Maguard 5626, as a disinfectant with bacterial and fungicidal activity against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 2-minute contact time:

Acinetobacter baumannii	MRID 489215-01 and 489215-04
Bordetella pertussis	MRID 489215-05
CA Methicillin Resistant *Staphylococcus aureus USA 400*	MRID 489215-09 and 489215-10
Escherichia coli	MRID 489215-11
Escherichia coli with extended beta-lactamase resistance	MRID 489215-15 and 489215-16
Klebisella pneumonia	MRID 489215-21 and 489215-22
Klebisella pneumonia Carbapenem Resistant	MRID 489215-23 and 489215-24
Proteus mirabilis	MRID 489215-25 and 489215-26
Pseudomonas aeruginosa	MRID 489215-27 through -29
Salmonella enterica	MRID 489215-33 and 489215-34

Staphylococcus aureus	MRID 489215-35 through -37
Vancomycin Intermediate Resistant *Staphylococcus aureus*	MRID 489215-39 and 489215-40
Vancomycin Resistant Enterococcus *faecalis*	MRID 489215-41 and 489215-47
Streptococcus pneumonia	MRID 489215-51
CA Methicillin Resistant *Staphylococcus aureus* USA 300	MRID 489215-52
Streptococcus pyogenes	MRID 489215-53
Methicillin Resistant *Staphylococcus aureus*	MRID 489215-54 and 489215-55
Candida albicans	MRID 489215-49 and 489215-50

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. In testing against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella enterica*, at least one of the product lots tested was at least 60 days old at the time of testing. Although only two lots of Samonella enterica were tested, in the original registration of this product, additional lots were tested at a dilution of 1:768 for 30 seconds with satisfactory results. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

5. The submitted efficacy data **support** the use of a 1:32 dilution of the product, Maguard 5626, as a disinfectant with fungicidal activity against *Trichopyton mentagrophytes* (MRID 489215-45 and 489215-46) on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a 2-minute contact time. Complete killing was observed in the subcultures. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

6. The submitted efficacy data **support** the use of a 1:768 dilution of the product, Maguard 5626, as a sanitizing rinse against the following microorganisms on pre-cleaned, hard, non-porous, food contact surfaces in the presence of 500 ppm hard water for a 30-second contact time:

Escherichia coli O103:K.:H8	MRID 489215-12
Escherichia coli O121:K-:H10	MRID 489215-13
Escherichia coli O45:K-:H-	MRID 489215-14
Escherichia coli O26:H11	MRID 489215-17
Escherichia coli O145:H28	MRID 489215-44

Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

7. The submitted efficacy data (MRID 489215-64) **support** the use of a 1:384 dilution of the product, Maguard 5626, as a sanitizing rinse against *Xanthomonas axonopodis* (citrus canker) on pre-cleaned, hard, non-porous, food contact surfaces in the presence of 500 ppm hard water for a 30-second contact time. Bacterial reductions of at least 99.999 percent were observed within 30 seconds. Neutralization confirmation testing met the acceptance criterion of growth within 1.0 $\log_{10}$ of the numbers control. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

8. The submitted data (MRID 489215-06 through 08) **support** the use of a 1:32 dilution of the product, Maguard 5626, as a disinfectant with bacterial activity against *Clostridium difficile*. Acceptable killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. Neutralization confirmation testing showed positive growth of the microorganisms. Viability controls were positive for growth. Purity controls were reported as pure. Sterility controls did not show growth.

9. The addition of *Salmonella enterica* serotype enteritidis to the label is approved for a dilution of 1:768 since the contact time on the revised label is 60 seconds (in the original submission for registration for Maguard 5626 this organism was on the label, but was removed at the request of EPA because the contact time was 30 seconds). No study was submitted for review, but the reviewer assumes that with the exception of the contact time, the MRID(s) that was previously reviewed was acceptable.

VII. LABEL

1. The proposed label claims that a 1:32 dilution of the product, Maguard 5626, is an effective disinfectant against the following viruses on hard, non-porous surfaces in the presence of 400 ppm hard water and 5% blood serum (a 100% organic load against Duck hepatitis B virus) for a 5-minute contact time:

Canine Parvovirus
Bovine Viral Diarrhea (Surrogate for Human Hepatitis C)
Duck Hepatitis B (Surrogate for Human Hepatitis B)

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that a 1:32 dilution of the product, Maguard 5626, is an effective disinfectant against the following viruses on hard, non-porous surfaces in the presence of 400 ppm hard water and 5% blood serum for a 2-minute contact time:

Adenovirus type 5
Norovirus
Murine Norovirus

These claims are acceptable as they are supported by the submitted data.

3. The proposed label claims that a 1:64 (Health Care Disinfectant #1) or 1:32 (Health Care Disinfectant #2) dilution of the product, Maguard 5626, is an effective disinfectant against the following viruses on hard, non-porous surfaces in the presence of 400 ppm hard water and 5% blood serum for a 2-minute contact time:

Herpes simplex virus type 2
Herpes simplex virus type 1
Influenza A
Rhinovirus type 37
Rotavirus
Respiratory Syncytial (RSV)

Vaccinia virus
Human immunodeficiency virus type 1

These claims are acceptable as they are supported by the submitted data.

4. The proposed label claims that a 1:64 dilution (Health Care Disinfectant #1) or a 1:32 dilution (Health Care Disinfectant #2) of the product, Maguard 5626, is an effective disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and 5% blood serum for a 2-minute contact time:

Acinetobacter baumannii
Bordetella pertussis
CA Methicillin Resistant *Staphylococcus aureus MRSA 400*
Escherichia coli
Escherichia coli with extended beta-lactamase resistance
Klebisella pneumonia
Klebisella pneumonia Carbapenem Resistant
Proteus mirabilis
Salmonella enterica
Pseudomonas aeruginosa
Staphylococcus aureus
Vancomycin Intermediate Resistant *Staphylococcus aureus*
Vancomycin Resistant Enterococcus *faecalis*
Streptococcus pyogenes
CA Methicillin Resistant *Staphylococcus aureus* MRSA 300
Streptococcus pneumonia
Methicillin Resistant *Staphylococcus aureus*
Candida albicans

These claims are acceptable as they are supported by the submitted data.

5. The proposed label claims that a 1:32 dilution of the product, Maguard 5626, is an effective disinfectant against *Trichopyton mentagrophytes* on hard, non-porous surfaces in the presence of 400 ppm hard water and 5% blood serum for a 2-minute contact time.

This claim is acceptable as it is supported by the submitted data.

6. The proposed label claims that a 1:768 dilution or 1:384 dilution of the product, Maguard 5626, is an effective sanitizing rinse against the following microorganisms on pre-cleaned, hard, non-porous, food contact surfaces in the presence of 500 ppm hard water for a 60-second contact time:

Escherichia coli O103:K.:H8
Escherichia coli O121:K-:H10
Escherichia coli O45:K-:H- (ECL 1001)
Escherichia coli O26:H11
Escherichia coli O145:H28
Salmonella enterica serotype enteritidis

This claim is acceptable as it is supported by the submitted data.

7. The proposed label claims that a 1:384 dilution of the product, Maguard 5626, is an effective sanitizing rinse against *Xanthomonas axonopodis* (citrus canker) on hard, non-porous surfaces in the presence of 500 ppm hard water and 5% blood serum for a 60-second contact time.

This claim is acceptable as it is supported by the submitted data.

8. The proposed label claims that a 1:32 dilution of the product, Maguard 5626, is an effective disinfectant against *Clostridium difficile* on hard, non-porous surfaces in the presence of 400 ppm hard water for a 2-minute contact time.

This claim is acceptable as it is supported by the submitted data.

9. The proposed label claims that a 3.5 fluid ounces of the product, Maguard 5626, per 10 gallons of water (or equivalent use dilution) at a rate of 1 quart per 1000 ft^3 **can be used as a fogger is not supported by efficacy data**. Fogging applications must be supported by efficacy data. **The registrant must delete fogging application claims**.

10. The following revisions must be made to the proposed label:

- Page 4: Delete the phrase "May be used as part of a comprehensive approach to *Clostridium difficile* spore control"
- Page 5: Delete statement "This product has been formulated to aid in the reduction of cross-contamination not only in hospitals, but in schools, institutions and industry."
- Page 5: Daily defense against (germs) (pathogens) appears to be a heighten efficacy claim.
- Page 7: What the registrant means by "Home Health Care (Settings)?
- Page 7: What is "patent chairs"? Does the registrant mean patient chairs?
- Page 8: Delete "Where housekeeping is of prime importance in controlling the hazard of cross contamination."
- Page 8: The registrant cannot have Optional Health Care Disinfection Sections for one label. What is "Health Care Disinfectant #1"?
- Page 9: The font size on "SPORICIDAL" must be changed. It appears everything on this page is under that heading. Disinfection of Animal Premises are not appropriate in this area.
- Page 10: There is a misspelling of *aureus* – the "s" is missing.
- Page 10: "What is Health Care Disinfectant #2" (2 to 5 minute Contact Time)? This is not appropriate. Change font size of Sporicidal.
- Page 12: What is "Health Car Disinfection #3" (10 minute Contact Time)?
- Page 12: What is the difference between "General Disinfection" (10 minute Contact Time) versus "Health Care Disinfectant #3" (10 minute Contact Time)? This is very confusing to the consumer.
- NOTE TO REVIEWER: Make sure all of the icons and symbols on page 17 have been approved.